UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION,
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: *Aspergillus flavus* AF36 use on corn.

TO: Shanaz Bacchus, M.S., Regulatory Action Leader
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511P)

FROM: Joel V. Gagliardi, Ph.D., Microbial Ecologist — signed: August 26, 2010
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511P)

THROUGH: John L. Kough, Ph.D., Senior Scientist — signed: August 26, 2010
Microbial Pesticides Branch, Biopesticides and
Pollution Prevention Division (7511P)

ACTION REQUESTED:
Review of efficacy data from 2008 EUP field testing of AF36 in Texas to support a section 3 label amendment for use on field corn.

CONCLUSION:
SUPPLEMENTAL – additional data from 2009 and 2010 Texas and Arizona field testing under the EUP that expires January 4, 2011 is necessary to evaluate this request.

DATA REVIEW RECORD

Active Ingredient:	*Aspergillus flavus* AF36.
Product Name:	AF36.
Company Name:	Arizona Cotton Research and Protection Council.
EPA Reg. Nos.:	71693-EUP-2.
Chemical Number:	006456.
Decision Number:	424829.
DP Barcode:	380006.
MRID Nos.:	479351-01.

BACKGROUND:
Aspergillus flavus AF36 is currently registered for use on cotton.

REVIEW SUMMARY:
Study Type: Product Performance Test Guidelines (OPPTS 810.1000); General Considerations for Public Health Uses of Antimicrobial Agents (OPPTS 810.2000 - *Draft*).
MRID Nos.: 479351-01.
Test Material: *Aspergillus flavus* AF36.
Study Summary: The EUP label indicates AF36 applications should be made prior to or during silking from late May through June. An amendment to the December 17, 2007 EUP issued May 27, 2009 extends the EUP until January 4, 2011 allowing use on 3,000 acres per year in Arizona and 5,000 acres per year in Texas. Data from 2008 use in Texas is included in the current submission - an application to amend the section 3 registration to include use on corn in addition to cotton. Some testing in Grayson County was carried out independently by grain elevator operators and contains different end-points than data submitted by cooperators. Grayson County data is from V7-10 treated corn, the only data from corn treated per label instructions. Cooperator data from Grayson County contains only means without identification to other field data from a total of 300 acres, while data from grain elevators varies on what details are reported with most of that data submitted containing aflatoxin measurements from untreated fields without determination of AF36 presence on the few treated fields. Data reported from Ellis, San Patricio, Wharton, Jackson, Victoria, Hidalgo and Medina Counties in Texas all included %AF36 and ppb aflatoxin on individual samples. None of these cooperators reported total treated and untreated acreage, corn cultivars used, distances between fields, yields or harvest dates. In all Counties other than Grayson application of AF36 was made later than label instructions directed, in some cases too late to have any meaningful effect on aflatoxin levels. Cyclopiazonic acid levels and %AF36 were reported from 36 treated and 25 control samples. One fifth of AF36 treated samples had measurable cyclopiazonic acid levels compared to none detected in control samples. Units for measured cyclopiazonic acid, and the corn stage where AF36 was applied, were not provided.
Classification: SUPPLEMENTAL – data from 2009 and 2010 from both Texas and Arizona under the EUP should be submitted and include the following; corn planting dates, AF36 treatment dates, stage of corn at treatment, conditions at and shortly after treatment (i.e. irrigation or rain events), acres represented by each sample, number of samples per plot, type(s) of corn planted, distance(s) between plots, harvest dates, %AF36 with aflatoxin and cyclopiazonic acid levels for each sample. Additional discussion should include whether nearby areas to treated and untreated plots have been treated with atoxigenic *Aspergillus flavus* AF36 or *Aspergillus flavus* NRRL 21882 (AflaGuard) previously for any testing or commercial purposes.

*** CONTAINS FIFRA CONFIDENTIAL BUSINESS INFORMATION ***

DATA EVALUATION RECORD

EPA Review by: Joel V. Gagliardi, Ph.D.

EPA Secondary Review by: John L. Kough, Ph.D.

Study Type	Product Performance Test Guidelines (OPPTS 810.1000); General Considerations for Public Health Uses of Antimicrobial Agents (OPPTS 810.2000 - *Draft*).
MRID No.	479351-01.
Test Material	*Aspergillus flavus* AF36.
Study Nos.	PR # 0378 B.
Sponsor	Arizona Cotton Research and Protection Council; 3721 E. Wier Avenue; Phoenix, Arizona 85040.
Testing Facility	Arizona Cotton Research and Protection Council; 3721 E. Wier Avenue; Phoenix, Arizona 85040.
Titles of Reports	Product Performance Data: Efficacy of AF36 in Corn.
Authors	Peter J. Cotty, Phillip J. Wakelyn, Michael Braverman, Larry Antilla.
Studies Completed	December 7, 2009.
Study Summary	The EUP label indicates AF36 applications should be made prior to or during silking from late May through June. An amendment to the December 17, 2007 EUP issued May 27, 2009 extends the EUP until January 4, 2011 allowing use on 3,000 acres per year in Arizona and 5,000 acres per year in Texas. Data from 2008 use in Texas is included in the current submission - an application to amend the section 3 registration to include use on corn in addition to cotton. Some testing in Grayson County was carried out independently by grain elevator operators and contains different end-points than data submitted by cooperators. Grayson County data is from V7-10 treated corn, the only data from corn treated per label instructions. Cooperator data from Grayson County contains only means without identification to other field data from a total of 300 acres, while data from grain elevators varies on what details are reported with most of that data submitted containing aflatoxin measurements from untreated fields without determination of AF36 presence on the few treated fields. Data reported from Ellis, San Patricio, Wharton, Jackson, Victoria, Hidalgo and Medina Counties in Texas all included %AF36 and ppb aflatoxin on individual samples. None of these cooperators reported total treated and untreated acreage, corn cultivars used, distances between fields, yields or harvest dates. In all Counties other than Grayson application of AF36 was made later than label instructions directed, in some cases too late to have any meaningful effect on aflatoxin levels. Cyclopiazonic acid levels and %AF36 were reported from 36 treated and 25 control samples. One fifth of AF36 treated samples had measurable cyclopiazonic acid levels compared to none detected in control samples. Units for measured cyclopiazonic acid, and the corn stage where AF36 was applied, were not provided.
Classification	**SUPPLEMENTAL** – data from 2009 and 2010 from both Texas and Arizona under the EUP should be submitted and include the following; corn planting dates, AF36 treatment dates, stage of corn at treatment, conditions at and shortly after treatment (i.e. irrigation or rain events), acres represented by each sample, number of samples per plot, type(s) of corn planted, distance(s) between plots, harvest dates, %AF36 with aflatoxin and cyclopiazonic acid levels for each sample. Additional discussion should include whether nearby areas to treated and untreated plots have been treated with atoxigenic *Aspergillus flavus* AF36 or *Aspergillus flavus* NRRL 21882 (AflaGuard) previously for any testing or commercial purposes.
Good Laboratory Practice	Not GLP Compliant – conducted using accepted scientific and/or commercial practices hence the study information is considered valid.

A. Efficacy Data for AF36 applied to corn:

The EUP label approved March 27, 2009 states "Apply *Aspergillus flavus* AF36 to the surface of the

soil under the plant canopy after last cultivation. Applications should be made prior to or during silking." Also "On corn, *Aspergillus flavus* AF36 has been shown to be effective when applied from late May through June." The specific claim to be evaluated is on the label, namely "*Aspergillus flavus* AF36 - For displacing aflatoxin producing fungi." An amendment to the December 17, 2007 EUP issued May 27, 2009 extends the EUP until January 4, 2011 allowing use on 3,000 acres per year in Arizona and 5,000 acres per year in Texas. Data from 2008 is included in the current submission. The registrant was specifically directed to include levels of aflatoxin and cyclopiazonic acid in treated and untreated corn.

Table 1: AF36® Efficacy studies with corn – Aflatoxin - 2008:

Farmer	Acres	Distance[1]	County	Stage	Yield[2]	Harvest	% AF36	Aflatoxin[3]
0 lbs / acre – Control								
[redacted]	50	North	GRAYSON, TX	V7-10	49			110
	21				56			115
	73				122			140
	23				101			130
	65				116			80
	132				111			180
	83				98			100
	101				93			16
	112				94			140
	105				100			87
	72				92			160
	92				92			78
	37				92			170
	34				108			190
	78				92			61
	28				94			14
Howe Com.	162				80			25
[redacted]	16				83			0
	225				89			40
Golden Cnr	53				86			41
[redacted]	22				98			55
	55				92			67
	55				109			140
	22				119			113
	182				101			37
		South >2mi				9/11/08		160
						9/11/08		3
						8/6/08		21
						9/8/08		560
						8/21/08		170
						9/5/08		1
						9/8/08		110
						9/19/08		110
						9/19/08		22

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[redacted]						9/11/08		110
						9/4/08		160
						9/15/08		100
						8/5/08		308
						8/6/08		330
						9/19/08		110
						9/5/08		140
						9/8/08		35
						8/13/08		35
						8/13/08		170
						8/6/08		800
						8/7/08		780
						8/8/08		984
						8/21/08		73
						9/19/08		178
						8/13/08		160
						8/1/08		100
						8/5/08		52
						9/19/08		78
						8/13/08		78
						8/5/08		500
						9/5/08		110
						9/8/08		110
						9/19/08		52
						9/11/08		140
Sears						9/5/08		52
Sears						9/8/08		52
[redacted]						9/11/08		160
						8/13/08		178
						9/11/08		24
						8/21/08		180
						9/11/08		3
						9/19/08		170
						8/5/08		74
						8/13/08		267
						9/5/08		140
						9/8/08		140
NTC-GC-SE						8/13/08		110
NTC-GC-SW						8/13/08		228
NTC-GC-EC						8/13/08		55
[redacted]		North	ELLIS, TX	V12-T			7	216
							18	66
							38	19
							7	131
							8	91
							13	184
							20	84

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[redacted]							0	68
958		South					14	89
961							14	306
5083							0	142
[redacted]							93	15
							93	21
							0	58
							0	139
							0	25
							0	33
Bayer			SAN PATRICIO, TX	Post VT 1-2 weeks			13	55
Bayer							8	15
Bayer							0	24
Bayer							0	8
[redacted]							0	23
							0	20
							0	15
							13	187
							33	23
2757-11258			JACKSON / WHARTON TX	Post VT 2-4 weeks			93	0
2758-10091							43	0
2757-11299							42	2
695-11920							24	2
02B							0	440
2160-11890							0	48
778-10477							0	17
778-10766							0	50
1903-11720							0	26
2197-11893							0	3
Dupont			VICTORIA, TX	Post R1			0	13
Dupont							6.67	40
Dupont							13.33	20
Dupont							7.69	27
Bayer							20.00	0
Bayer							0.00	0
Bayer							0.00	17
Bayer							9.09	0
[redacted]							15.38	0
							7.69	21
							14.29	nd
							0.00	0
99-BCR			HIDALGO, TX	Post VT >4 weeks			0	6
99-BCR							7	36
M-14a							0	33
M-14b							60	32
M-14c							0	194
M-15a							91	7

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M-15b							0	223
6+7 TJa							100	32
6+7 TJb							8	11
28a							0	8
28b							8	13
34a							0	64
34b							7	29
34c							0	31
[redacted]		≤2 mi.	MEDINA, TX	Not reported			57	0
							92	22
							92	0
							60	0
Airport							67	9
Airport							93	5
Airport							67	5
Airport							77	27
[redacted]							0	35
	10 lbs / acre – AF36®							
	71	North	GRAYSON, TX	V7-10	109			0
	79				119			0
NTC-Test		South				8/21/08		9
NTC-Test						8/21/08		22
NTC-Test						8/21/08		0
NTC GC-EC						8/13/08		24
[redacted]		North	ELLIS, TX	V12-T			80	60
							73	39
							87	0
							80	61
							87	25
							93	26
							92	0
							100	0
							87	69
							100	39
							43	0
860		South					100	8
864							93	27
865							100	58
933							100	14
953							82	64
958							100	2
961							83	27
[redacted]			SAN PATRICIO, TX	Post VT 1-2 weeks			100	25
							0	4
							27	64
							73	54
							0	14

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[redacted]							88	22
Bayer							87	5
Bayer							100	5
Bayer							87	6
Bayer							93	15
[redacted]							7	1
							100	0
							73	4
							0	6
							100	2
							100	0
							100	0
10407			JACKSON / WHARTON TX	Post VT 2-4 weeks			87	1
12076							53	47
11908							46	1
11284							45	1
11305							42	2
10099							38	0
01A							33	1
02A							21	1
695-17A							20	0
778-10645							13	3
778-10636							8	0
778-10417							7	0
778-10638							7	0
2160-23070							7	12
778-10422							7	3
1903-10443							0	1
2197-10626							7	1
778-10405							0	17
Dupont			VICTORIA, TX	Post R1			50.00	7
Dupont							84.62	16
Dupont							100.00	46
Dupont							100.00	2
Bayer							60.00	34
Bayer							71.43	34
Bayer							100.00	6
Bayer							53.85	3
[redacted]							21	0
							86	1
							73	6
							100	20
M-11a			HIDALGO, TX	Post VT >4 weeks			54	93
M-11b							58	38
9MCa							100	31
9MCb							77	96
9MCc							100	9

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2+3 TJa							79	35
2+3 TJb							50	1
2+3 TJc							0	3
25a							100	0
25b							82	22
25c							100	5
47WBCa							0	63
47WBCb							0	45
47WBAc							0	76
[redacted]			MEDINA, TX	Not reported			92	19
							80	20
							60	28
							86	7
							100	0
							100	0
							69	1
							100	0
							100	15
							100	15
							100	30
							100	0
BBN Hwy 90							100	1
BBN Hwy 90							93	1
BBN Hwy 90							100	2
BBN Hwy 90							80	26
[redacted]							100	18
							93	21
							100	18

[1] In some cases an approximate distance from control to treatment plot(s) was reported, though in most cases this data is absent; [2] Field corn cultivar and in some cases yield and harvest dates are supplied, though not consistently; [3] Aflatoxin (ppb) by commercial ELISA, samples with readings over 80ppb are diluted and re-run. Samples in bold were treated according to EUP label instructions.

Table 2: FDA Action levels for poisonous or deleterious substances - AFLATOXIN	
Commodity	**Action Level (ppb)**
Animal Feeds	
Corn and peanut products intended for finishing (i.e., feedlot) beef cattle	300
Cottonseed meal intended for beef, cattle, swine, or poultry (regardless of age or breeding status)	300
Corn and peanut products intended for finishing swine of 100 pounds or greater	200
Corn and peanut products intended for breeding beef cattle, breeding swine, or mature poultry	100
Corn, peanut products, and other animal feeds and feed ingredients but excluding cottonseed meal, intended for immature animals	20

Corn, peanut products, cottonseed meal, and other animal feed ingredients intended for dairy animals, for animal species or uses not specified above, or when the intended use is not known	20
Brazil nuts	20
Foods	20
Milk	0.5 (aflatoxin M1)
Peanuts and Peanut products	20
Pistachio nuts	20

RESULTS AND DISCUSSION:

The data submitted was not all part of the intended EUP. Rather, most data from Grayson County was obtained independently by grain elevator operators who sampled mostly untreated corn arriving at their elevators. Data from North Grayson County included the cooperator name, FSA#, acres sampled, corn variety, yield in bushels/acre and ppb aflatoxin. North Grayson County had two treated fields and 25 untreated fields. In South Grayson County a harvest date and approximate distance of untreated fields from treated fields was also supplied, but not number of acres, corn variety or yield data. South Grayson County had four treated fields and 49 untreated with 3 untreated fields on the same farm as treated fields. None of these fields reported AF36 presence for any assay. A summary table on page 12 in MRID 479351-01 lists %AF36 from 17 treated samples in North and 16 treated samples in South Grayson County, compared to 8 control samples, though individual data is absent. Table 2 on page 12 of MRID 479351-01 states these samples are from corn treated at V7-10 on a total of 300 acres but there is no included comparison to location of treated and untreated acreage sampled by the grain elevators so the summary data from cooperators is inconclusive. Grayson County data does show a significant effect of AF36 colonization and aflatoxin reduction generally, though individual cooperators, fields, corn cultivars, or distribution of acreage among treated and non-treated plots for reported means from cooperators was not submitted.

Data reported from Ellis, San Patricio, Wharton, Jackson, Victoria, Hidalgo and Medina Counties in Texas all included %AF36 and ppb aflatoxin on individual samples. None of these reported treated and untreated acreage, corn cultivars, distance between fields, corn yields or harvest dates. In all instances outside Grayson County application of AF36 was later than label instructions directed, in some cases too late to have any meaningful effect on aflatoxin levels.

Cyclopiazonic acid levels and %AF36 were reported from 4-7 samples each in Ellis, Grayson, Hidalgo, San Patricio and Victoria Counties in Texas and compared to control samples. Cyclopiazonic acid was detected in 7 of 36 AF36 treated corn samples and in none of the 25 control samples. Units of measured cyclopiazonic acid was not reported and ranged from 0.8-1.4 for a mean of 0.3 across all AF36 treated samples. It was not reported at what corn stage AF36 was applied in these cases.